Systematical studies on Anthidiini (Hymenoptera: Megachilidae): A geometric morphometric approach

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Abstract: The classificatory schema in some groups of Megachilidae reorganized by means of shape differences in terms of the inter- and intra-specific and inter- and intra-generic and subgeneric deformations. The relative warps, UPGMA trees and PCA graphs obtained from 30 landmarks were shown. Both *Anthidium cingulatum* Latreille and *A. florentinum* (Fabricius) should better classify in the same subgenus *Anthidium* s. str. In the other case under the results of shape variation our results supported the hypothesis that *Paraanthidium* as a subgenus of *Anthidium* Fabricius. On the other hand *Icteranthidium* Michener and *Paraanthidiellum* Michener showed enough dissimilarity to be kept as separate genera. Our analysis also supported the monophyletic origin of Megachilini which was clustered as a separate group by all methods used.

Key words: Megachilidae; Anthidiini; systematics; geometric morphometrics; UPGMA; generalized procrustes analysis; relative warps

1 INTRODUCTION

Resembling most of the other groups in entomology, there is still no consensus among researchers about the classification of some groups in Megachilidae systematics (Warncke , 1980; Özbek and Zanden , 1993; Gogola , 1999; Michener , 2000). Possible reasons for this confusion may be the lack of sufficient morphological characters or the existence of artificial intermediate taxa. However , these problems were partially solved by means of changes made in the basic characters leading to changes in the place of some genera (Michener , 2000).

The tribe Anthidiini also experiences similar systematical confusion mentioned above. The tribe can be differed from the others in terms of the following features: Shortness of stigma and pterostigma; the existence of yellow, white or red maculations on integument; having cleft claws in females or having basal articulation; the existence of the second recurrent vein in the distal of the second submarginal cell; the lack of dorsal lamella of metapleura; the lack of preapical transverse flange or carina in T6 of the males

(Banaszak and Romasenko , 1998; Michener , 2000). The earliest study on the phylogeny was carried out by Müller (1996). Despite the whole variation in the male genitalia, Warncke (1980) classified all the genera of Anthidiini which are not parasitic in the genus Anthidium Fabricius, 1804. However, the taxa which were considered as subgenera in this study were treated as genera level by Tkalcu (1966), Banaszak and Romasenko (1998), Gogola (1991, 1999). Özbek and Zanden (1993) regarded Paraanthidium Friese, 1898 as a subgenus of Anthidium Fabricius, 1804 and Paranthidiellum Michener, 1948 as a subgenus of Pseudoanthidium Friese, 1898. Michener and Griswold (1994) and Michener (2000) viewed Paraanthidium Friese, 1898 and Archianthidium Mavromustakis, 1939 as subgenera belonging to the genus Trachusa Panzer, 1804 and he regarded the Paranthidiellum Michener, 1948 as the synonymous of the Pseudoanthidium Friese, 1898.

The lack of consensus in the systematical studies directed several authors toward the seach for alternative approaches (Mayr and Ashlock , 1991). The DNA based techniques and cladistic methodology in time partially solved some of these problems. The use of

geometric morphometrics in understanding the process underlying the biodiversity is a relatively new methodology. After the big revolutionary change in the 1990's , this approach began to represent an important new paradigm for the statistical study of "shape" in biology and other fields of science (Rohlf, 1999a; Rohlf, 2002). In the last ten years, geometric morphometric citations per year have increased considerably (Lynch, 2004). Especially the landmarkbased morphometrics has gained significant support among scientists including anatomists (Pavlinov, 2001; Hennessy and Stringer, 2002; Lockwood et al., 2002) and entomologists (Alibert et al., 2001; Kligenberg, 2003). Unlike the analytical approaches, the geometric one is aimed at comparison of the shapes themselves (Pavlinov , 2001). Its high level of explanation power on the shape and size variation and co-variation among the organisms, provided an alternative solution especially for problematic groups in systematics and ecology (Aytekin et al., 2003).

The principles of these methods are based on capturing the two- or three-dimensional Cartesian coordinates of landmarks which are basically the homologous points among the individuals that have been previously assigned the same names (Bookstein, 1991). Differences among individual configurations of landmarks can be translated to several mathematical functions which fit the differences (Alibert et al., 2001). Then the Procrustes distance (the square root of the sum of the summed squared distances between homologous landmarks) can be used as a metric for comparing shapes (Rohlf, 1999a). The registered landmark configurations (k landmarks and dimensions) can be represented as points in a shape space which is of lower dimensionality than the figure space since location, rotation and scale differences have been removed (O'Higgins and Jones, 1999). For twodimensional data, the space of Procrustes registered specimens is therefore of dimensionality (Bookstein , 1991; O'Higgins and Jones , 1999; Rohlf , 1999a). This space is known as Kendall's shape space (Kendall, 1984) which is however non-Euclidian. But it possibly can allow the study of well known multivariate analysis (Sneath and Sokal, 1973) like obtaining a principal components analysis (PCA) or unweighted pair-group method using arithmetic averages (UPGMA) phenogram if it is superimposed orthogonally (or stereographically) in the tangent plane to Kendall's shape space (Dryden and Mardia, 1998). In addition, as these methods preserve the geometrical relationships among landmarks, they also provide a powerful vision for visualizing deformations in the shape of the original specimen in means of displacement vectors deformation grids as warps (Rohlf, 1993; Alibert et al., 2001). Some structures like the insect wings with

the least degrees of freedom are one of the most appropriate structures for such studies (Pavlinov , 2001). Together with warps , PCA of Procrustes residuals and UPGMA phenograms can provide an excellent combination of techniques for the two purposes of such studies as we made for Anthidiini here: First to detect and then to describe the differences among taxa (Lockwood *et al.* , 2002).

2 MATERIALS AND METHODS

2.1 Study area and sampling

92 specimens were used from the Megachilidae collection in Hacettepe University which were sampled between 1999 and 2003 from different eco-regions of Middle Anatolia (Fig. 1). The study area (totally 178.080 km²) has generally a steppe character with Astragalus sp., Acantholimon sp., Centaurea sp., Echium sp., and Anchusa sp. There are mountains and hills (altitude ca. 1800 m) that covered mainly by Pinus nigra Am. ssp. pallasiana (Lamb) Holmboe, different species of Quercus sp., Picea sp. and Juniperus sp. with Cedrus libani A. Richard (Davis, 1968 – 1985). Agriculture and urbanization with high industry is also common in most places where collections were made. Right front wings, right hind wings and heads (from frontal view) of the females of Anthidium cingulatum Latreille, 1809, 12 Anthidium florentinum (Fabricius, 1775), 15 Icteranthidium laterale Latreille , 1809 , 15 Megachile maritima (Kirby, 1802), 16 Paraanthidium 1781) interruptum (Fabricius , and Paraanthidiellum lituratum (Panzer, 1801) were prepared for morphometric analysis. maritima which is from Megachilini was used as controlgroup for the analysis (not as reference). Megachilini were preferred because the tribe Osmiini was understood to be paraphyletic with respect to Megachilini (Engel, 1999). Diagnose of the species and nomenclature followed that proposed by Banaszak and Ramasenko (1998) and Warncke (1980). All the specimens were screened at the time they were collected for the presence of known ecto- and/or endo-parasites to shield the morphometric data from possible traumatic variations (Mayr and Ashlock , 1991; Aytekin et al. , 2002). The body parts were then removed from each specimen with forceps and mounted in entellane on labelled slides. All slides were photographed using a Leica MZ-7.5 (r) stereoscopic zoom dissection microscope with a DC-300 digital camera system, digitized, and archived with special information codes added. From the 92 specimens some of them were eliminated because of the problems during preparation procedure and finally 92 specimens for front wings , 91 (one specimen from P. interruptum is ignored) specimens for hind wings and

specimens were digitized twice (Arnqvist and Mårtensson, 1998). The second session of measurement was conducted after the specimens had been removed and replaced under the microscope in order to take the positioning error into account (Arnqvist and Mårtensson, 1998; Alibert et al., 2001). No analogous systems were used during the whole procedure.

3 RESULTS

3.1 Relative warps

By using an orthogonal alignment projection method data obtained from front wings, hind wings and heads were analyzed by means of the first two relative warps. For the front wings, relative positions of the average configurations of the species A. cingulatum, A. florentinum and P. interruptum were clustered together where P. lituratum and I. laterale formed another group while M. maritima separated from rest of all in the shape space defined by the first two relative warps (x = 1, y = 2, $\alpha = 0$) (Fig. 5). The deformation grids of the front wing landmark configuration showed that the out-group M. maritima have a typical shape difference than the other taxa (Fig. 6). (Eigenvalue was calculated as 4.788 for the first relative warp). Whereas Anthidium spp. and Paraanthidium interruptum have similar front wing shapes when they are superimposed on the mean shape configuration. Same methodology also used for the hind wings and heads. We have got similar configuration for Anthidium spp. and Paraanthidium interruptum on the hinwings. Relative positions of the average configurations of the species A. cingulatum, A. florentinum and P. interruptum were linearly arranged

where P. lituratum and M. maritima formed another group while I. laterale separated from rest of all in the shape space defined by the first two relative warps (x =1, y = 2, $\alpha = 0$) (Fig. 7). The deformation of the hindwings for A. florentinum and P. interruptum were more similar especially on the landmarks 5 and 6 (Fig. 8) (Eigenvalue was calculated as 2.107 for the first relative warp). This is also determined on the head shape. Relative positions of the average configurations of the species A. cingulatum, A. florentinum, I. laterale and P. interruptum were clustered together where P. lituratum and M. maritima separated from rest of all in the shape space defined by the first two relative warps (x = 1 , y = 2 , $\alpha = 0$) (Fig. 9). The similar orientation of the landmarks 1, 3, 4, 5 and 6 showed typically for Anthidium spp. Paraanthidium (Fig. 10). (Eigenvalue was calculated as 2.330 for the first relative warp).

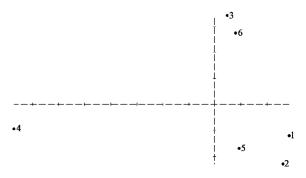


Fig. 5 Relative positions of the average configurations of the species (1:A. cingulatum; 2:A. florentinum; 3:I. laterale; 4:M. maritima; 5:P. interruptum; 6:P. lituratum) for Anthidiini and Megachilini in the shape space defined by the first two relative warps (x=1, y=2, $\alpha=0$). Data from front wings. Filled points indicate the species.

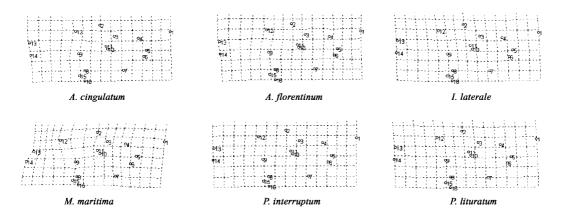


Fig. 6 Deformation grids of the six species of Anthidiini and Megachilini used in the present study superimposed on the reference configuration (x = 1, y = 2, $\alpha = 0$) in relative warps. Data from front wings. Open circles with numbers indicate the landmarks.

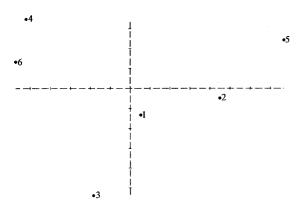


Fig. 7 Relative positions of the average configurations of the species (1:A. cingulatum; 2:A. florentinum; 3:I. laterale; 4:M. maritima; 5:P. interruptum; 6:P. lituratum) for Anthidiini and Megachilini in the shape space defined by the first two relative warps (x=1, y=2, $\alpha=0$). Data from hind wings. Filled points indicate the species.

3.2 PCA

When a PCA is conducted by using the data obtained from the shape of the front wings, hind wings

and heads we reached similar results. The species were ordered along the first two principal components similar to front wings (Fig. 11) and hind wings (Fig. 12) data. The PCA of head data gave relatively sparse distribution than the ones obtained from the wings (Fig. 13).

3.3 UPGMA

The UPGMA phenogram formed by SAHN clustering obtained from the data of whole landmarks (60 characters) used in the study showed distinct group patterns for all taxa (Sneath and Sokal, 1973). 80 specimens were randomly used for the cluster formation. The control-group M. maritima clustered separately from the rest of the taxa. I. laterale and P. lituratum also clustered as a distinct group which indicates not only the species difference but also enough for identification of the genus level. A. florentinum, A. cingulatum and P. interruptum showed enough distinct patterns for being different species group but showed a mixed structure for a different genus category level (Fig. 14).

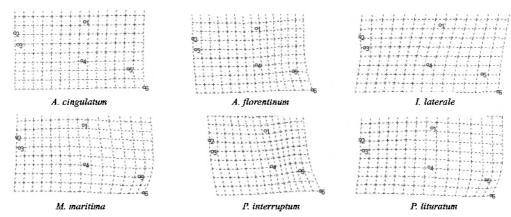


Fig. 8 Deformation grids of the six species of Anthidiini and Megachilini used in the present study superimposed on the reference configuration (x = 1, y = 2, $\alpha = 0$) in relative warps. Data from hind wings. Open circles with numbers indicate the landmarks.

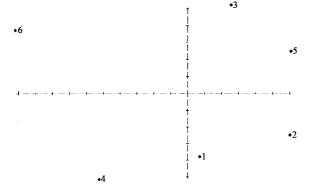


Fig. 9 Relative positions of the average configurations of the species (1: A. cingulatum; 2: A. florentinum; 3: I. laterale; 4: M. maritima; 5: P. interruptum; 6: P. lituratum) for Anthidiini and Megachilini in the shape space defined by the first two relative warps (x = 1, y = 2, $\alpha = 0$). Data from heads. Filled points indicate the species.

4 DISCUSSION

The research field of geometric morphometrics is a diversity of morphological structures which may be uncertain individual variation; differences among discrete groups like taxa, sexes, ages, insect castes, biomorphs, ecotones and others (Pavlinov, 2001) or sometimes fluctuating asymmetry (Klingenberg, 2003). Therefore we hypothesized that the classificatory schema in some groups of Megachilidae would be reorganized by means of shape differences in terms of the inter and intra-specific and inter and intra-generic and subgeneric deformations.

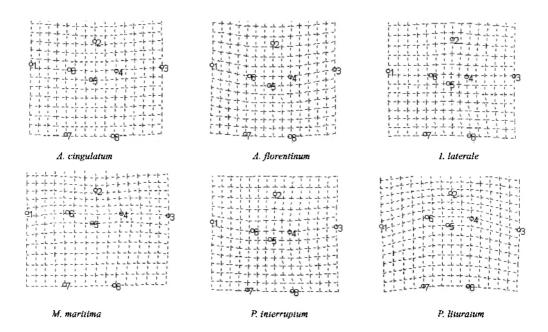


Fig. 10 Deformation grids of the six species of Anthidiini and Megachilini used in the present study superimposed on the reference configuration (x = 1, y = 2, $\alpha = 0$) in relative warps. Data from heads. Open circles with numbers indicate the landmarks.

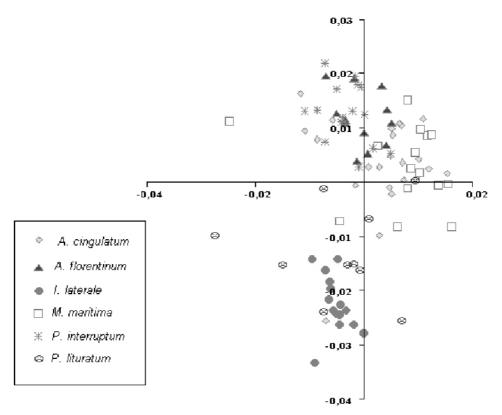


Fig. 11 Distribution of the six different species of Anthidiini and Megachilini. Principal component analysis conducted for the 16 landmarks digitized from the front wings.

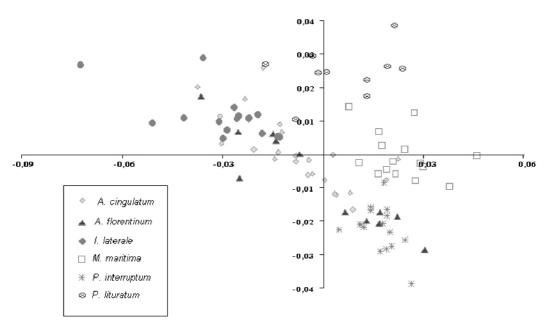


Fig. 12 Distribution of the six different species of Anthidiini and Megachilini. Principal component analysis conducted for the 6 landmarks digitized from the hind wings.

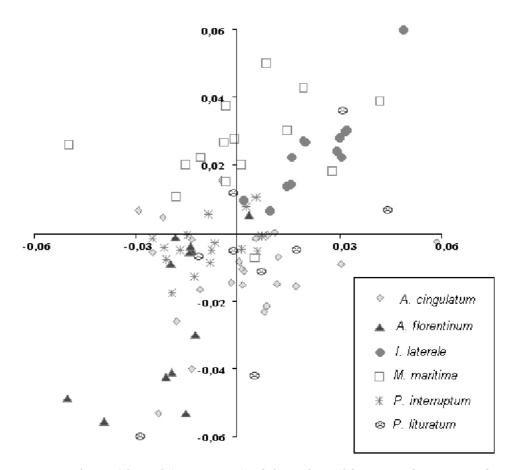


Fig. 13 Distribution of the six different species of Anthidiini and Megachilini. Principal component analysis conducted for the 8 landmarks digitized from the heads.

The results obtained from the relative warps of a total of 30 landmarks from three main body parts showed that A. cingulatum, A. florentinum and P. interruptum did not show enough differences in the bending energies of the grid deformations. Therefore it is better not to classify those in separate genera. The inter-specific variations were basically in landmarks 10 and 11 in front wings. This information can indicate a close irrelativeness but clearly need additional data especially from DNA sequence. The general interspecific variations were in landmarks 2,3 and 5 in hind wings while and 4,5 and 6 in head shape.

The patterns of covariance in the landmark coordinates after Procrustes superimposition were studied with PCA (Dryden and Mardia , 1998; Klingenberg , 2003). The extracted first two PC's from our analysis had a clear geometric explanation in Anthidiini. Especially the data obtained from the wings supported our hypothesis. Relatively sparse distribution pattern from the head data would possibly due to the landmark types , measurement error and most degrees of freedom (Readers unfamiliar with the techniques may be directed to Bookstein (1991) and Zelditch *et al*. (2004) for details). The UPGMA phenogram also clearly gave the same schema.

These results are however different from those of proposed by Warncke (1980). Both A. cingulatum and A. florentinum showed separate species character but should better classify in same subgenus as Anthidium s. str. In the other case P. interruptum should better not classified as a separate genus. Under the results of shape variation we would propose Paraanthidium as a subgenus of Anthidium Fabricius, 1804. On the other hand Icteranthidium and Paraanthidiellum showed enough dissimilarity to be kept as separate genera. This classification supported the approach of Özbek and Zanden (1993). Our analysis also proved the monophyletic origin of Megachilini which was clustered as a separate group by all methods used.

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References

- Alibert P, Moureau B, Dommergues JL, David B, 2001. Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. *Zoologica Scripta*, 30(4):299–316.
- Amqvist G, Mårtensson T, 1998. Measurement error in geometric morphometrics: emprical strategies to assess and reduce its impact on measures of shape. *Acta Zoologica Academia Scientiarum Hungaricae*, 44:73–96.
- Aytekin AM, Çağatay N, Hazīr S, 2002. Parasites, micro-organisms and floral choices in natural populations of bumblebees (Apidae: Hymenoptera) in Ankara province. *Turkish Journal of Zoology*. 26.

- 149 155.
- Aytekin AM, Rasmont P, Çağatay N, 2003. Molecular and morphometric variation in *Bombus terrestris lucoformis* Krüger and *Bombus terrestris dalmatinus* Dalla Torre (Hymenoptera: Apidae). *Mellifera*, 3(6):34–40.
- Banaszak J , Romasenko L , 1998. Megachilid Bees of Europe. Pedagogical University of Bydgoszcz. 239.
- Bookstein FL, 1991. Morphometric Tools for Landmark Data. Geometry and Biology. Cambridge University Press. 435.
- Davis PH , 1968 1985. Flora of Turkey and the East Aegean Islands. Volume 1-9. University of Edinburgh Press , Edinburgh.
- Dryden IL , Mardia KV , 1998. Statistical Shape Analysis. London: John Wiley and Sons.
- Engel MS, 1999. *Megachile glaesaria* the first Megachilid bee fossil from amber (Hymenoptera: Megachilidae). American Museum Novitates. No: 3 276. 13pp.
- Gogola A , 1991 . Contribution to the Knowledge of the Bee Fauna of Slovenia (Hymenoptera: Apidae). Scopolia , 25:1-33.
- Gogola A , 1999. Bee Fauna of Slovenia: Checklist of Species (Hymenoptera: Apoidea). Scopolia , 42:1-79.
- Hennessy RJ, Stringer CB, 2002. Geometric morphometric study of the regional variation of modern human craniofacial form. American Journal of Physical Anthropology, 117:37-48.
- Kendall DG , 1984. Shape manifolds , procrustean metrics and complex projective spaces . Bull . Lond . Math . Soc . , 16:81-121 .
- Klingenberg CP, 2003. Developmental instability as a research tool: using patterns of fluctuating asymmetry to infer the developmental origins of morphological integration. In: Polak M ed. Developmental Instability, Causes and Consequences. Oxford University Press. 427 442.
- Lockwood CA , Lynch JM , Kimbel WH , 2002. Quantifying temporal bone morphology of great apes and humans: an approach using geometric morphometrics. *Journal of Anatomy* , 201(6):447 464.
- Lynch JM , 2004. D'Arcy: An online bibliography of landmark-based morphometrics. http://www.public.asu.edu/~jmlynch/geomorph/index. html.
- Mayr E , Ashlock PD , 1991. Principles of Systematic Zoology. 2nd ed. McGraw-Hill , Inc. 475 pp.
- Michener CD, 2000. The Bees of the World. The Johns Hopkins Universitive Press. Baltimore and London. 913 pp.
- Michener CD , Griswold TL , 1994. The classification of old world Anthidiini (Hym., Megachilidae). *The University of Kansas Science Bulletin* , 55 (9): 299 327.
- Müller A , 1996. Host-plant specialization in western paleartic anthidiine bees (Hymenoptera: Apoidea: Megachilidae). Ecological Monographs , 66 (2):235 – 257.
- O'Higgins P , Jones N , 1999. Morphologika. Tools for shape analysis. Software. University College London.
- Özbek H, van der Zanden G, 1993. A preliminary review of the Megachilidae of Turkey Part Ⅲ. The Anthidiini (Hymenoptera: Apoidea). Türk. Entomol. Derg., 17(4):193 207.
- Pavlinov IY, 2001. Geometric morphometrics, a new analytical approach to comparision of digitized images. In: Information Technology in Biodiversity Resarch: Abstracts of the 2nd International Symposium. St. Petersburg: Russian Academy of Sciences. 41 90.
- Rohlf FJ, 1993. Relative warp analysis and an example of its application to mosquito wings. In: Marcus LF, Bello E, Garcia-Valdecasas A eds. Contributions to Morphometrics. Museo Nacional de Ciencias Naturales (CSIC), Vo. 8. Madrid, Spain. 131 159.
- Rohlf FJ , 1999a. On the use of shape spaces to compare morphometric methods. *Hystrix* , 11(1):1-17.
- Rohlf FJ, 1999b. Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification*, 16:197-223.
- Rohlf FJ , 2000. Ntsys-Pc Version 2.1. Numerical taxonomy and multivariate analysis system. Exeter Software.
- Rohlf FJ , 2002. Geometric morphometrics in phylogeny. In: Forey P , Macleod N eds. Morphology , Shape and Phylogenetics. Francis & Taylor: London. 175 – 193.
- Rohlf FJ , 2004a. tpsDIG. Version 1.40. N.Y.: Software. State University Sunny at Stony Brook.
- Rohlf FJ, 2004b. tpsUTIL. Version 1.28. N.Y.: Software. State University Sunny at Stony Brook.
- Rohlf FJ , 2004c. tpsRELW Version 1.34 N.Y.: Software. State University Sunny at Stony Brook.

Sneath PHA , Sokal RR , 1973 . Numerical taxonomy . San Francisco : W. H. Freeman & Co .

Tkalcu B, 1966. Revision of some Latreille's species of the tribe Anthidini with the description of a new species (Hymenoptera: Apoidea: Megachilidae). *Acta Ent. Bohemoslov.*, 63:62-66.

Warneke K , 1980. Die Bienengattung Anthidium Fabricius , 1804 in der

Westpalaarktis und im Turkestanischen Becken. Entomofauna Zeitschrift Für Entomologie, 1(10):119–209.

Zelditch ML, Swiderski DL, Sheets HD, Fink WL, 2004. Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, New York. USA, 443 pp.

黄斑蜂族的系统学研究:几何形态测量方法

(膜翅目:切叶蜂科)

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摘要:利用采集于土耳其安纳托利亚中部(Middle Anatolia)的切叶蜂科6个种92头标本选取虫体上的30个特征点(landmarks)(其中前翅16个后翅6个,头部8个)利用UPGMA等数值分类方法对不同属和种的亲缘关系进行了探讨。据此对一些种的归属和一些属的划分提出了一些与传统分类观点相同或不同的见解,但各种分析方法的结果都支持切叶蜂族Megachilini起源的单系性。

关键词:切叶蜂科;黄斑蜂族;系统分类学;几何形态测量;UPGMA

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